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SOLUBLE SILICON (Si) AS POTENTIAL TREATMENT OF POST HARVEST DECAY CAUSED BY *Fusarium proliferatum*

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ABSTRACT

Fusarium proliferatum is one of the common causal agents for pre-harvest and post-harvest diseases of plants. This study examined the effectiveness of applying soluble silicon as an alternative treatment in controlling post-harvest disease. *In vitro* assay was performed by applying poison plate technique with three different Si concentrations; 1.5% Si (v/v), 2.5% Si (v/v) and 3.5% Si (v/v) whereas for *in vivo* assay, *F. proliferatum* was inoculated into mature and healthy produce. The cucumber and aubergine were selected for *in vivo* assay and stored at 27-28°C for 8 days after treatment to assess disease severity. Results showed that Si significantly inhibited mycelial growth of *F. proliferatum in vitro* and the 3.5% (v/v) of Si was more effective than 1.5% (v/v) and 2.5 (v/v) as it showed a greater percent inhibition of radial growth. Therefore, 3.5% Si (v/v) was chosen for *in vivo* assay. The soluble Si demonstrated a lower disease severity on both, cucumber and aubergine. However, *F. proliferatum* is less severe on cucumber (15% decaying) than aubergine (33% decaying). This suggests that the Si may inhibit the germination of fungal spores and the elongation of their germ tubes. There is a potential role of soluble silicon as a decay-control product and could help reduce wastage especially fruit in storage.

Key words: Soluble silicon, *Fusarium proliferatum*, inhibit, *in vitro* and *in vivo*

INTRODUCTION

The fungal contamination along with pest infection of agricultural production caused a severe problem in developing countries when there are high losses of major food and crops yield (Agrios, 2005). *Fusarium* is considered as one of the most important groups of fungi, not only of its diversity but the ability to cause serious plant diseases such as vascular wilts, fruit and root rots on various types of crops and vegetables (Bruton & Duthie, 1996; Nur Ain Izzati *et al.,* 2009; Siti Nordahliawate *et al.,* 2012). Thus, *Fusarium* species are known as the ubiquitous soil borne plant pathogens in terms of economic damage in agricultural productions all over the world (Bentley *et al.,* 2006).

The degree of post-harvest loss of fruits and vegetables also has been a growing concern in the food industry in recent years. In 2011, Food and Agriculture Organization of United Nations (FAO) has mentioned that post-harvest losses of fresh produce represent a critical component affecting global food losses. After harvesting, vegetables contain relatively high microorganism which lead to spoilage and plant pathogenic fungi that making worst of infection (Barth *et al*., 2009). Subsequently, this may lead to short shelf-life and less acceptability of the produce. These include vegetables belong to the solanaceae and cucurbitaceae families (Snowdon, 1990; Tournas, 2005; Naureen *et al.,* 2009).

In order to meet the increasing of food demand, post-harvest losses must be reduced without increasing the burden on the natural environment

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ensuring future global food security. Therefore, application of synthetic fungicides as the primary means of controlling post-harvest diseases is now has been progressively restricted, due to increasing concerns on the protection of the environment, human health and increased of pathogen resistance to fungicides (Dianz *et al.,* 2002; Marín *et al.,* 2003; Ma *et al.,* 2004; Rial-Otero *et al.,* 2005).

Other alternatives control is needed to replace the synthetic fungicides such as application of silicon (Si). Silicon creates a physical barrier which can restrict fungal hyphae penetration and may induce accumulation of antifungal compounds such as flavonoid and diterpenoid phytoalexins which can degrade fungal and bacterial cell walls (Alvarez & Datnoff, 2001; Brescht *et al.,* 2004). Silicon could provide a non-hazardous strategy for disease control; as an alternative to fungicides to reduce the potential environmental threat to land and water (Liu *et al.,* 2009).

Numerous studies have shown that silicon (Si) is effective in controlling several fungal diseases in plants (Fauteux *et al.,* 2005; Datnoff *et al.,* 2007; Van Bockhaven *et al.,* 2013). The silicon (Si) increases the resistance in rice against leaf and neck blast, sheath blight, brown spot, leaf scald and stem rot (Rodrigues & Datnoff, 2005) and provide an effective action in controlling powdery mildew in cucurbits (Samuels *et al*., 1991; Menzies *et al*., 1992; Belanger *et al*., 2003). In China, combination of silicon product, sodium metasilicate with antagonist yeasts; *Cryptococcus laurentii* and *Rhodotorula glutinis* was applied to jujuba fruit and fungal pathogen such as *Penicillium expansum* and *Alternaria alternate* in fruit wounds were inhibited (Tian *et al.,* 2005). There was also a positive effect of silicon on yeasts against *Penicillium expansum* when being applied to apples (Farahani *et al.,* 2012). Little work has been undertaken on the effect of bioactive silicon on post-harvest treatment. Hence, the objective of this study is to examine the utility of applying soluble silicon (Si) as an alternative post-harvest treatment.

MATERIALS AND METHODS

Source of pathogen and inoculum preparation

Fusarium proliferatum was obtained from Fungal Culture Collection, Laboratory for Pest Disease and Microbial Biotechnology (LAPDiM), Universiti Malaysia Terengganu. *Fusarium* species confirmed as causal organism of Fusarium fruit rot was grown onto Potato Dextrose Agar (PDA) to confirm its viability. Spore suspension was prepared from 10-day-old PDA cultures incubated at 25ºC. The *Fusarium* cultures were flooded with sterile distilled water and gently dislodge the spore using a glass rod. The spore suspensions obtained were passed through four layers of sterile cheesecloth to remove mycelial fragments (Lane *et al*., 2012). The number of spores in the suspension was observed with a haemocytometer and concentration of spores was adjusted to 3 x 10^6 conidia/mL using sterile distilled water.

In vitro assay

The effect of Si on mycelial growth of the pathogens was assayed by using poison plate technique (Fiori *et al.,* 2000). Treatments at different concentrations; 1.5% Si (v/v), 2.5% Si (v/v) and 3.5% Si (v/v) were tested against *F. proliferatum*. Each treatment was performed in three replicates. The soluble silicon was added into autoclaved PDA, mixed by shaking gently and poured into petri plates. After media solidified as poison plate, 5-mm diameter disc from seven days old *Fusarium* cultures was plucks out and placed at the centre of poison plates in aseptic condition. Plugs of culture also placed onto media plates without silicon as control. All plates were incubated for 6 days at 28 ± 2°C and radial growth of colony was measured every two days. The two readings in the control and treatments were transformed into percent inhibition of radial growth by using the following formula (Skidmore & Dickenson, 1976):

$$\text{Percentage inhibition} = \frac{(C–T)}{C} \times 100$$

Where,
C = colony diameter (mm) growth in the control
T = colony diameter (mm) growth in the treatment

In vivo assay

Produce from two different families were choose for *in vivo* assays which are cucumber (cucurbitaceae) and aubergine (solanaceae). Healthy and mature produce with no mechanical injury were selected. Fruit was dipped for 2 min into soluble silicon (Si) containing 0.01% (v/v) Tween 20, and the controls were treated only with 0.01% (v/v) Tween 20 and air dried. Wounds were made on the healthy produce by using steriled fine needle. The samples were inoculated by spraying with spore suspension of 3 x 10^6 conidia/mL. All inoculated samples were placed in a plastic container as a damp chamber and incubate at ambient temperature (27–28°C). Three replicates were made for each treatment and disease assessment was conducted in every 2 days. Disease severity was based on 0–4 scales with a slight modification, 0=no disease symptom or decay on the fruit, 1=1–10% decaying, 2=11–20% decaying, 3=21–30% decaying and 4=30% decaying (Illeperuma & Jayasuriya, 2002). Data was analysed by using SPSS version 20.0; Mann-Whitney U test.

RESULTS AND DISCUSSION

Effect of Si on *Fusarium proliferatum* mycelial growth

Results showed that the mycelial growth of *Fusarium proliferatum* on the poison plate (PDA mixed with soluble silicon) was significantly slower with increasing of silicon concentration (Fig. 1). After 6 days, the percent inhibition of radial growth of *F. poliferatum* was increased with the increase in concentration of soluble silicon; 52%, 61% and 69% for 1.5% Si (v/v), 2.5% Si (v/v) and 3.5% Si (v/v), respectively. We observed that *F. poliferatum* colony mature faster in control (without silicon) compared to other treatments (Fig. 1A and B). The soluble silicon can cause reduction in cell turgor pressure of *Aspergillus alternata, F. semitectum*, and *Trichoderma roseum,* which resulted in collapse and shrinkage of hyphae and spores (Yang *et al.,* 2006). Therefore, treatment with silicon can cause inability of fungi to sporulate *in vitro*.

Effect of Si on inoculated produce; cucumber and aubergine

Result showed that treatment with 3.5% Si (v/v) significantly reduced the disease severity of *F. proliferatum* on inoculated cucumber and aubergine compared to control (Fig. 2A and B). However, *F. proliferatum* was more severe on aubergine than cucumber; on day 8, aubergine showed 33% of decaying and cucumber showed only 15% of decaying. The same positive result showed on Chinese cantaloupes that were dipped into the solution of sodium silicate and had less severe of post-harvest pink rot (Guo *et al.,* 2006). Silicon application of silicon oxide and sodium silicate had also reduced the infection rate of *Fusarium* spp., decreased fruit mass loss (%) and decay of fruit compared to the control of melon (Liu *et al.,* 2009). Furthermore, enzyme such as peroxidase was observed in sodium silicate-treated melons that may reduce post-harvest decaying (Liu *et al.,* 2009).

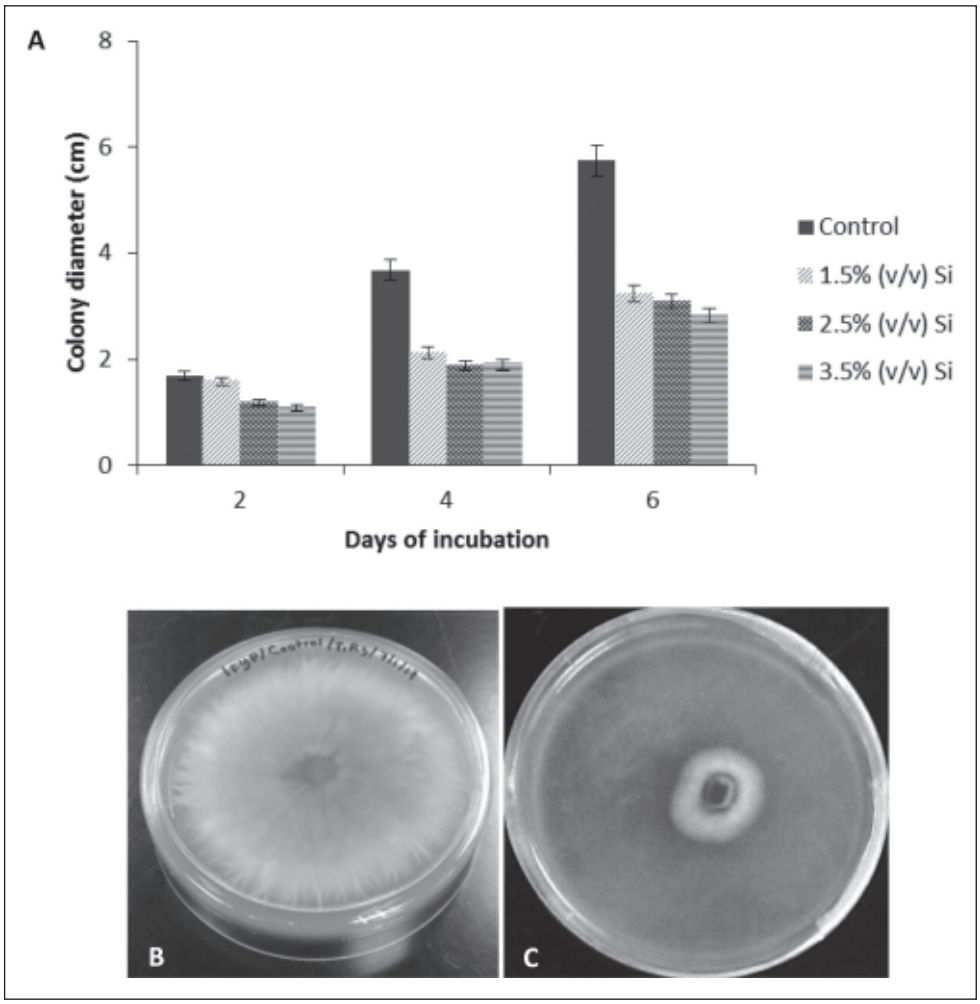

Fig. 1. Effect of silicon on colony diameter (mm) of *Fusarium proliferatum* grown in poison plate agar (PDA mixed with soluble silicon) during 6 days of incubation (A) and morphology of *F. proliferatum* (B) grown in PDA without silicon compared to (C) grown in poison plate agar.

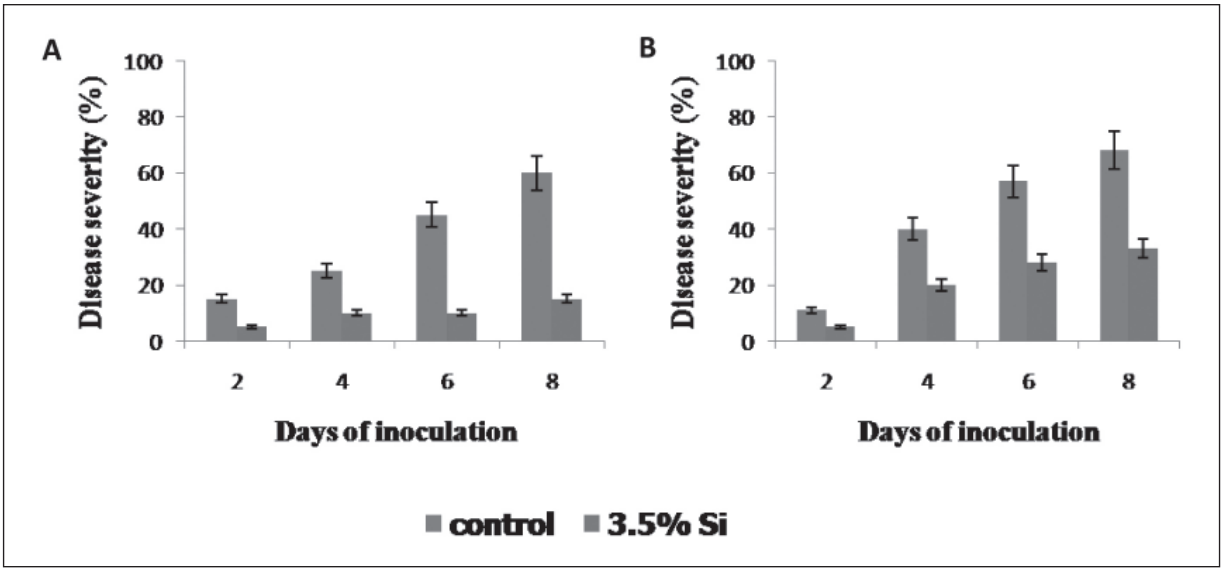

Fig. 2. Percentage of disease severity of (A) cucumber and (B) aubergine treated with 3.5% Si (v/v) and without silicon treatment (control) incubated for 8 days at ambient temperature in a damp plastic container.

There may be a possibility of involving the physical barrier formation for the disease reduction in fruits subjected to Si treatments compared to control when delaying the penetration of germinating fungal spores. Previous study had showed that the percentage numbers of appressoria of *Colletotrichum gloeosporioides* were higher on tomatoes being treated with silicon compared to controls (Bailey *et al.,* 1992). This happened when the fungus penetration process was delayed; the number of appressoria increased on the surface of the tissue which implies the presence of a physical barrier over the surface of fruit peel that impeded the process of infection (Bailey *et al.,* 1992).

In addition, silicon application in post-harvest had been recommended for increasing storage life and maintaining fruit quality because of more phenylalanine ammonia-lyase and total phenolic activities that enhance the fruits ability to resist stressful cold conditions (Habibi, 2015). This also suggested that Si may be involved in modulating enzymes when there was an increasing of flavonoids and phenolics in response to high concentrations of Si (Mditshwa *et al.,* 2013). Moreover, postharvest studies on apricot and avocado have proved Si to be a safe and effective antioxidant source (Habibi, 2015; Tesfay *et al.,* 2011).

CONCLUSION

We concluded that soluble silicon could retard the growth of *Fusarium proliferatum*. Both *in vitro* assay as well as application on the healthy cucumber and aubergine showing slows of fungus growing or decaying. In addition, efficacy was also influenced by the concentration of Si. Until now, fungicides have been most commonly used to control postharvest diseases but the chemical residues and pathogen resistance to chemical are critical issues. Thus, the uses of soluble silicon may replace the application of fungicide or other washing agent of post-harvest and to control the postharvest fungal disease. Although the application of soluble silicon and mechanism involved Si resistance of plants to fungus is not yet fully understood but our result showed that silicon may be considered as a promising ingredient of coating agent especially in delaying the growth of fungi and potentially as a decay-control product as well as preservative. Subsequently, improved post-harvest characteristics in storage, processing and distribution.

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